Maple Sirup. XVIII.

1619

Bacterial Growth in Maple Sap Collected with Plastic Tubing

(Manuscript received April 10, 1961)

H. A. Frank and C. O. Willits
Eastern Regional Research Laboratory,[a]
Philadelphia 18, Pennsylvania

SUMMARY

Study was made of the growth of normal, adventitious microflora of maple sap during collection with transparent plastic tubing by methods currently used. Early in the season an aerial system yielded sap higher in bacterial counts than did a ground system, which counts may have contributed to premature stoppage of sap flow. A build-up of pockets of infection, resulting from the coils of tubing used to anchor suspended tubing lines, seems to contribute to the build-up of bacterial population in the aerial collection system. In the ground system, taphole vents contributed to a more rapid infection than did unvented tapholes.

LARGE systems of interconnected plastic-tubing lines to collect and transport maple sap from taphole to collection tank or storage tank, are replacing less efficient methods used for several generations (Frank and Willits, 1960; Laing *et al.*, 1960; Morrow, 1958). These systems eliminate much of the cost and labor of collection with buckets, and have two additional advantages. First, extraneous matter (leaves, twigs, or insects) is kept from getting into the sap. Second, the polyvinyl tubing used is sufficiently transparent to the ultraviolet radiation in sunlight to permit germicidal action on organisms present in the sap (Frank and Willits, 1960). Nevertheless, new problems arise.

One of the chief difficulties of plastic-tubing systems is mechanical interference with sap flow from "vapor locks" that form in the lines. "Vapor locks" are static pockets of gas liberated from the taphole during sap flow or of air drawn into the system. Unless these pockets are eliminated, sap flow may be greatly reduced or even stopped completely.

Another, and more important, problem is microbial growth in the sap within the tapholes and in the tubing. Microbial populations, especially the psychrophilic bacteria, frequently increase to high levels between sap flows, when static bodies of sap remain in the system (Edson *et al.*, 1912; Frank and Willits, 1960; Naghski and Willits, 1953, 1955; Sheneman and Costilow, 1959; Sheneman *et al.*, 1959). These reservoirs of infection can contaminate the entire system.

Maple sap is sterile when it issues from a freshly made taphole (Edson, 1910; Willits, 1958). As the sap-collection season progresses, the microbial population increases in the taphole tissue and in the sap (Edson, 1910; Hayward and Pederson, 1946; Holgate, 1950; Sheneman and Costilow, 1959; Willits, 1958). The early microflora of sap is composed largely of bacteria, predominantly gram-negative psychrophilic rods (Edson, 1910; Edson *et al.*, 1912; Hayward and Pederson, 1946; Sheneman and Costilow, 1959). Later, yeasts, and then molds, are present in appreciable numbers. The deleterious effects of excessive microbial growth in maple sap on the color and flavor of maple sirup has been reported by many investigators (Edson, 1910; Edson *et al.*, 1912; Hayward and Pederson, 1946; Holgate, 1950; Hucker and Pederson, 1942; Naghski *et al.*, 1957; Willits, 1958). In addition, excessive microbial growth in the taphole tissue can cause premature stoppage of sap flow (Naghski and Willits, 1953; Sheneman *et al.*, 1959), reducing the sap crop. When microbial growth does occur, considerable debris (organic matter) is deposited on the inner surface of the tubing and on spouts and other fittings. Disinfection of contaminated surfaces and removal of debris is laborious but necessary (Willits *et al.*, 1959).

At present, two types of plastic-tubing installations are being used for collecting and transporting maple sap: an aerial and a ground system.

The aerial system consists of suspended plastic-tubing lines connecting, in series, a large number of tapholes on several trees (often as many as fifty). Sap from the most remote taphole passes successively through all spouts in the series before leaving the exit line. To maintain tension the tubing is anchored to a groove in the spout, using a coil arrangement. Sap in this coil does not drain easily, and may serve as a static harbor of infection for subsequent sap runs.

In the ground system the plastic-tubing installation consists of vertical pieces of tubing connecting the taphole spout to a conduit on the ground. Sap does not flow from one taphole through another, as in the aerial system.

A study was made of the growth of the normal, adventitious microflora of maple sap that collects in transparent plastic-tubing lines with the methods currently used by many maple-sirup producers.

METHODS

Observations were made during the 1959 and 1960 maple sap seasons at a sugar bush near Ambler, Pennsylvania. The trees were tapped with an aseptic technique described previously (Naghski and Willits, 1955), and then flushed with a hypochlorite disinfectant diluted to 5000 ppm available chlorine. Washed and disinfected plastic fittings and tubing (Willits *et al.*, 1959) were installed to plan.

In 1959, parallel aerial and ground systems of eight tapholes each were installed on five trees. Neither line was vented. Samples were collected from the effluent sap whenever a sap run occurred. Microbial populations were estimated by diluting the sample and plating with tryptone-glucose-yeast extract agar. Colonies were counted after incubation for about 40 hr at 81°F, a period too short for colony development of yeasts

[a] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

and molds encountered in sap. We therefore assume that the counts represent bacterial populations only.

All the subsequent obesrvations were in 1960. The plastic-tubing systems were installed as described except for the modifications given below. Aliquots of sap, suitable for estimating bacterial counts, were obtained from the sampling apparatus installed adjacent to each taphole (Fig. 1). Samples were obtained by first removing the protective test tube and then opening the clamp to allow the sap to drip freely from the open tube for several minutes. A sterile test tube was attached, and the sap sample collected. This tube was removed, and the protective test tube reinstalled. Bacterial populations in the sap samples were estimated as described above.

The aerial system consisted of 2 tapholes per tree on a row of 5 trees. Tapholes were drilled at gradually decreasing heights to facilitate gravitational flow of sap in one direction. Taphole heights ranged from about 7 feet, on the first tree (I), to about 3.5 ft, on the fifth tree (V). A vent consisting of a 3-ft piece of tubing was connected to the highest taphole on tree I and suspended vertically upward. Samplers (Fig. 1) were installed at each spout so that sap could be collected as it issued from the taphole. The tubing between the trees was kept taut by anchoring a coiled portion of the tubing through a groove in the taphole spout. The tubing was also supported by metal wire lines suspended between the trees to help keep sags at a minimum.

In the ground system, installed on a row of 6 trees on a gradual slope, all tapholes were made at breast height.

Two trees were used to study the microbial population in sap collected with multiple tapholes. Each tree had 3 tapholes, connected in series by 2 short pieces of plastic tubing, emptying into a single effluent line connected to the collection conduit line. The sampler on each tree was installed just below the taphole connected to the effluent line. Thus, sap samples were composites of sap output of all tapholes on each tree. One tree had no venting system. The other tree was vented by a 3-ft piece of tubing connected to the topmost taphole and extending vertically upward.

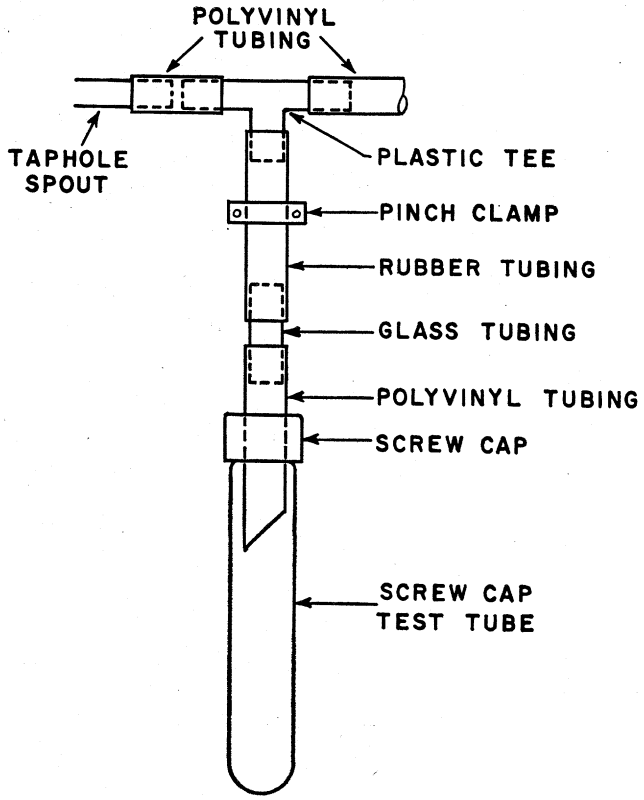

Fig. 1. Apparatus for sampling sap collected with plastic tubing.

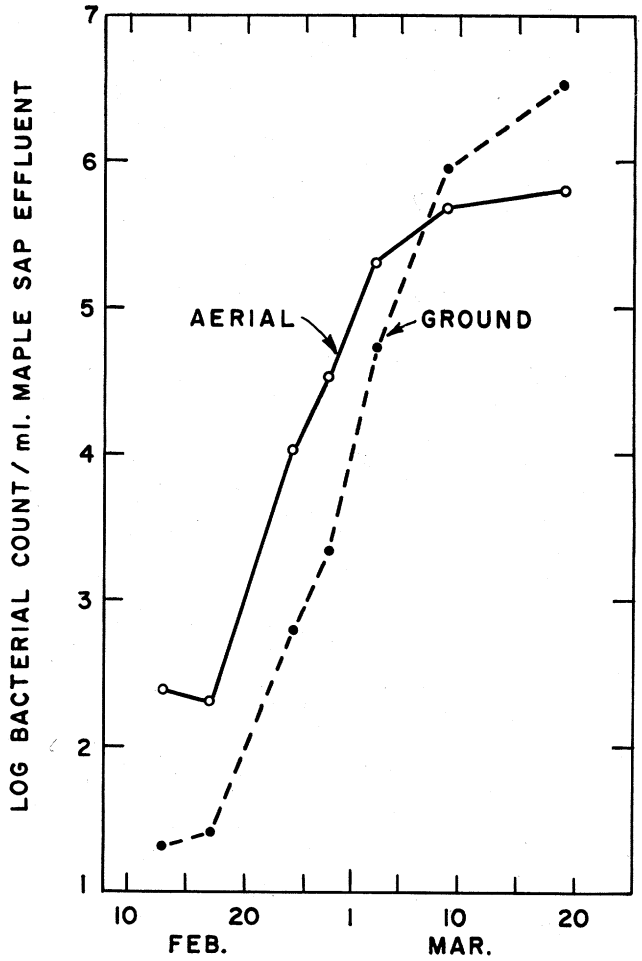

Fig. 2. Comparison of bacterial populations in effluent maple sap from ground and aerial plastic-tubing collection systems.

The 9 tapholes on the 4 remaining trees were connected by individual lines to the collection conduit line. Of these, 5 were equipped with vent lines and 4 were not. All 9 tapholes were installed with samplers for collection of sap.

When sap did not flow from a taphole while other tapholes yielded sap, the stoppage was considered to have resulted from excessive microbial growth (Naghski and Willits, 1953; Sheneman *et al.*, 1959).

RESULTS

Preliminary observations in 1958 had indicated that microbial growth had taken place in experimental aerial and ground sap-collection systems. Since these systems were being used primarily for a study of their mechanical function in sap collection, no estimates were made of microbial populations in the sap. However, considerable microbial debris was found occluded to the plastic fittings and one the inner surfaces of the tubing. Sap samples collected late in the season contained several million organisms per milliliter.

The 1959 systems were installed on January 14. The first collection was on February 13 (Fig. 2). During the earlier runs, microbial populations were slightly higher in sap collected by the aerial system (Fig. 2). As the weather became warmer, the difference between the sap in both systems narrowed, and in fact became reversed on the last two runs.

All subsequent data in this paper are from observations made in 1960. Trees were tapped and tubing installed on February 2, 1960.

Fig. 3 shows the bacterial counts in sap collected from the 5 trees installed with the aerial system of plastic tubing. It is apparent that samples collected from tapholes progressively further "downstream" show increasing bacterial counts. Tree

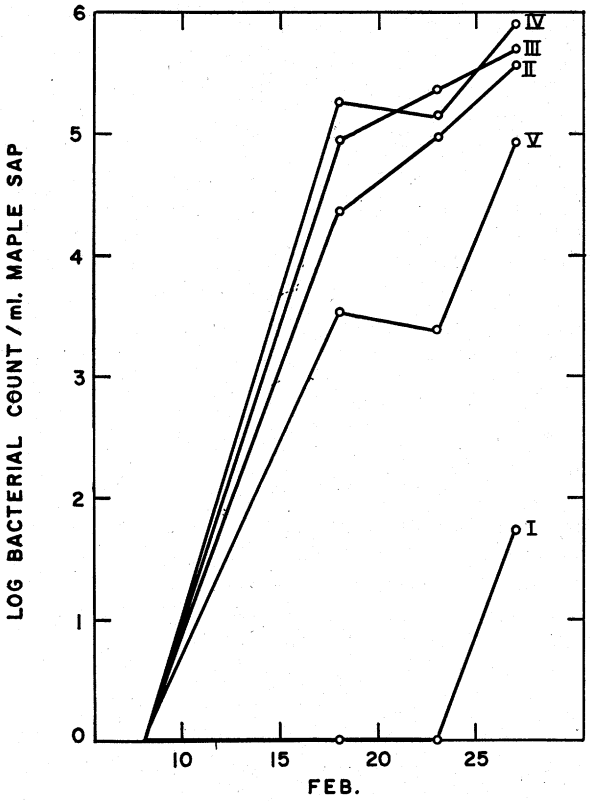

Fig. 3. Comparison of bacterial populations in maple sap from the five tapholes of the aerial plastic-tubing collection system (taphole I being the first, and taphole V the last, in the series).

V, on the other hand, does not fall in line with the trend exhibited by the data observed with trees I through IV, because no coiling of the tubing was used on tree V. The significance of this difference in anchoring is covered in the Discussion.

Fig. 4 shows the effect of venting on a ground-system installation composed of multiple tapholes connected in series on individual trees. Venting apparently permits earlier contamination of the system, leading to a higher bacterial count than in similar but unvented ground systems. As the weather becomes warmer the discrepancy between the two systems decreases and the microbial counts become more nearly equal.

The effect of venting on individual tapholes is best seen in Fig. 5, which demonstrates that bacterial counts rose more quickly, and to higher levels, in vented tapholes than in unvented tapholes.

Observations of stoppage of sap flow are difficult to pinpoint with respect to the exact day when sap flow ceased. Actually, one can detect sap flow stoppage in a taphole only when weather conditions exist that cause sap flow in other tapholes. Thus, one can only say on which day sap last flowed. At the beginning of the 1960 season, a sap run was good from all tapholes. For 19 days after February 28, the weather was cold; consequently, sap did not flow again until March 17. On that date none of the aerial-system tapholes were running. In contrast, only one of four unvented tapholes in the ground system had stopped running, and two of five vented holes.

DISCUSSION

As the season progresses and the weather warms, microbial growth rates accelerate, especially at night, and the population in sap is not entirely destroyed. Nevertheless, the effect of sunlight as a disinfecting agent is appreciable, and has also been demonstrated in polyvinyl plastic bags (Naghski and Willits, 1953). The end result of this inhibition of microbial growth in sap is sirup of a higher quality (i.e., lighter color). Since the spouts and tapholes are protected from contamination, the collecting season is correspondingly lengthened and the total sap crop increased.

This report is from preliminary observations made in the field during two maple seasons, 1958–59, and from experiment in 1960. The initial observation of the microbial population in maple sap collected with plastic tubing (Fig. 2) demonstrated that the systems were subject to entry of microorganisms, probably around the spout or other fittings and through handling. In the installation studied in 1960, the vent was a 4-ft piece of tubing leading vertically from the taphole to a point up on the tree. The aerial system, comprising 10 tapholes, had only a single vent, at the taphole furthest from the exit end of the tubing. The ground system, composed of individual tapholes feeding directly into larger conduits, was vented with a spout having two tubulations, one connecting with the conduit and the other with the vent tube.

Originally it was planned to test several methods of venting during the 1960 study to evaluate the degree to which contamination may enter via this system. Some vent lines were installed without any protection over the exposed open end; others were protected with either a small wad of cotton or were covered with a "Kapette" (plastic protective pipette cover manufactured by Nelson-Kapell, Inc., Los Angeles; mention of this name does not imply endorsement of this company by the Department of Agriculture over any other similar companies not named). However, dur-

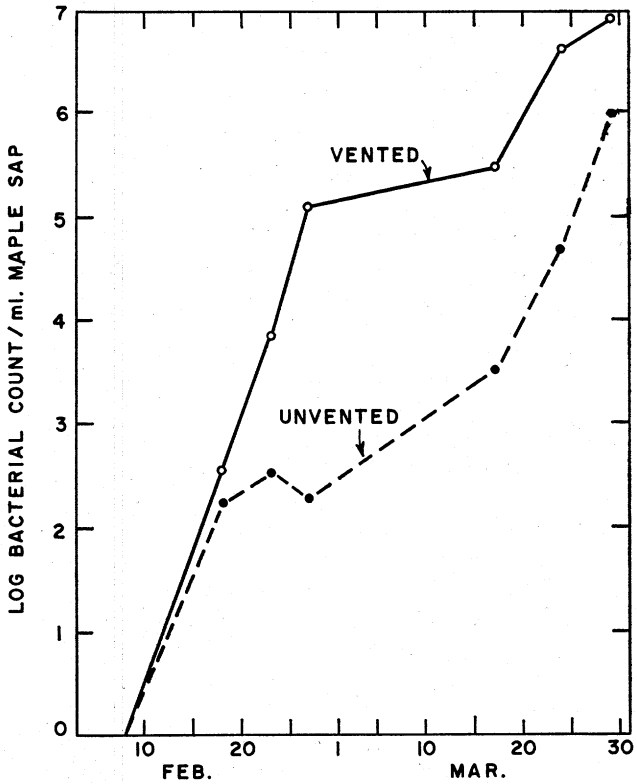

Fig. 4. Comparison of bacterial populations in maple sap collected with the ground tubing system using vented and unvented multiple tapholes connected in series.

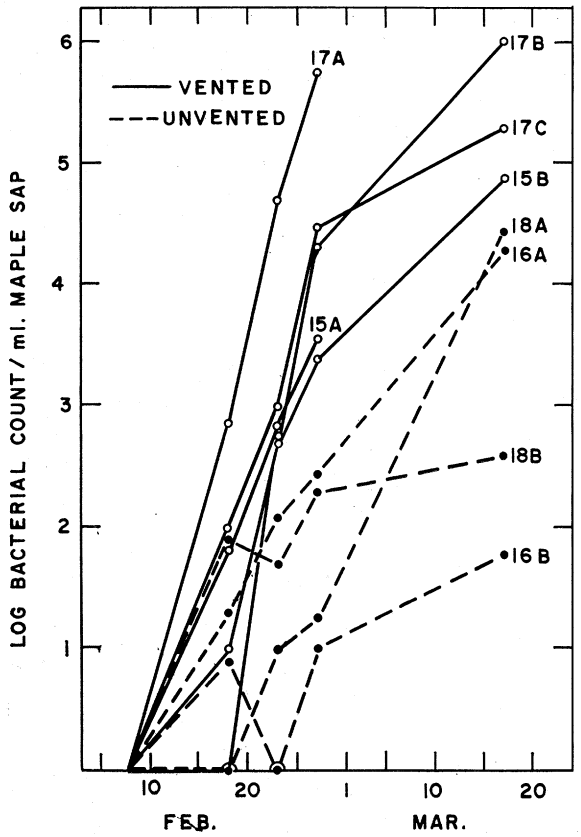

Fig. 5. Comparison of bacterial populations in maple sap from vented and unvented individual tapholes of the ground plastic-tubing collection system. Numbers identify the tree, and the letters identify the tapholes.

ing the first sap run we noted that imperfections in the vent systems prevented free escape of gas from the taphole. As a result, sap was being carried up the vent lines with the gas bubbles, and even running out the top of the vents. The Kapettes and cotton plugs became moist or were literally forced out of the tubing. Consequently, we removed all protection from the vent lines. Currently, work is being carried out to develop a spout that will separate sap from the entrapped gas bubbles and allow free escape of gas so that none of the sap will be carried into the vent lines. When completed, vented lines in this new system could be protected to keep out aerial contaminants.

It was hoped that some of our observations would elucidate the path of infection in the aerial system. The data in Fig. 3 are not clear-cut in this regard, however. The bacterial populations in sap coming from tapholes I through IV show a pattern of increase that might be expected if the more-downstream tapholes are being continuously fed with infected sap. This conclusion might seem unwarranted because taphole V, which is the most downstream of all tapholes and therefore should have the highest degree of infection, is rather low in bacterial count. The explanation, we feel, is fairly straightforward. The plastic lines connecting trees I through IV were suspended by anchoring the tubing to a groove in the spout, and then it was coiled and attached to the spout. The coil of tubing provided a pocket of sap that, during periods of non-flow, would serve as a "breeding place" for microbial buildup. Tree I had only one such coil, since it connected to only one tree (II); other trees in the line were connected to two trees. Tree V was the exception, since no coils were used on it. Instead, the line was anchored by wrapping around the tree, thus eliminating the static pocket of sap provided by the coil; this suggests an explanation for the lower bacterial count.

The use of multiple tapholes connected in series on a single tree emptying into a single line was studied since it provides a simpler means of collecting sap from a large tree without the expense of connecting the individual tapholes of each tree to the conduit. Fig. 4 shows that bacterial populations in vented systems of multiple tapholes connected in series are higher than similar systems not vented. The effects of venting individual tapholes (Fig. 5) are consistent with the effects shown in Fig. 4—that venting leads to earlier contamination of the sap.

The stoppage of sap flow tends to indicate that the build-up of taphole microflora is faster in the aerial system than in the ground system, whether vented or not. It is unfortunate that weather did not allow sap to flow between February 27 and March 17. Additional data in that period might well have shown that the aerial tapholes did not stop flowing simultaneously, but rather in accordance with their level of contamination, with trees V and I being the most long-lived.

That venting is desirable was apparent from observations on sap flow. Without exception, vented tapholes ran more freely than did unvented tapholes. It is not unreasonable to expect that some modification permitting free escape of gases will be forthcoming. If so, preventing or decreasing contaminants entering via the vent should be possible.

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